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LONG-TERM CHANGES IN AMPHETAMINE-INDUCED REINFORCEMENT AND AVERSION IN RATS FOLLOWING EXPOSURE TO ^{56}Fe PARTICLE

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ABSTRACT

Exposing rats to heavy particles produces alterations in the functioning of dopaminergic neurons and in the behaviors that depend upon the integrity of the dopaminergic system. Two of these dopamine-dependent behaviors include amphetamine-induced reinforcement, measure using the conditioned place preference procedure, and amphetamine-induced reinforcement, measured using the conditioned place preference procedure, and amphetamine-induced aversion, measured using the conditioned taste aversion. Previous research has shown that exposing rats to 1.0 Gy of 1GeV/n ^{56}Fe particles produced a disruption of an amphetamine-induced taste aversion 3 days following exposure, but produced an apparent enhancement of the aversion 112 days following exposure. The present experiments were designed to provide a further evaluation of these results by examining taste aversion learning 154 days following exposure to 1.0Gy ^{56}Fe particles and to establish the convergent validity of the taste aversion results by looking at the effects of exposure on the establishment of an amphetamine-induced conditioned place preference 3, 7, and 16 weeks following irradiation. The taste aversion results failed to confirm the apparent enhancement of the amphetamine-induced CTA observed in the prior experiment. However, exposure to ^{56}Fe particles prevented the acquisition of amphetamine-induced place preference at all three-time intervals. The results are interpreted as indicating that exposure to heavy particles can produce long-term changes in behavioral functioning.

INTRODUCTION

When the biological endpoint of interest in studies on the effects of exposure to heavy particles is DNA strand breaks or mutagenesis, one factor that must be taken into account is the possibility of repair of the radiation-induced damage (cf., Tobias, 1985; Curtis, 1986). In contrast, repair at the cellular level is not a significant factor when the tissue of concern is the neuron. Thus, initial studies showed no recovery in oxotremorine-enhanced dopamine release in rats tested six months following exposure to ^{56}Fe particles (Hunt et al., 1989; Joseph et al., 1992). However, despite the fact that there may be little or no tissue recovery following exposure of the CNS to heavy particles, there is the possibility of behavioral recover. The concept of "recovery of function" refers to observation of the ability of an organism to perform a behavioral task, which had been lost following destruction of a portion of the brain. In effect, undamaged neural tissue is capable, at least to some limited extent, of supporting the behavior that was originally mediated by the part of the brain that was damaged. As such, the long-term effects of exposure to heavy particles on behaviors mediated by the CNS must be directly examined in order to determine whether or not there are additional changes in behavior as a function of time since irradiation.

Previous research has examined the effects of exposure to 1.0 Gy of 1GeV/n ^{56}Fe particles on two dopamine-mediated behaviors: the amphetamine induced conditioned taste aversion (CTA) and the amphetamine-induced conditioned place preference (CPP). A CTA is produced when a toxic unconditioned stimulus is paired with a normally preferred conditioned stimulus (e.g. 10% sucrose), such that the organism will avoid ingestion of that conditioned stimulus at a subsequent presentation. As such, the CTA is a measure of the capacity of a compound to

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initiate a change in behavior in response to changes in the internal environment. The CPP is produced by pairing a potentially rewarding stimulus with a unique environment, such that, when allowed to choose, the organism will spend more time in the environment that has been paired with the rewarding stimulus. As such, the CPP is a measure of the capacity of the organism to approach stimuli (e.g., drugs of abuse, food, sex, etc.) that can reinforce behavior.

In a previous series of experiments (Rabin et al., 1998, 2000a), it was found that exposing rats to 1.0 Gy of ^{56}Fe particles disrupted the acquisition of a conditioned taste aversion (CTA) produced by injection of amphetamine (3 mg/kg, ip) 3 days following irradiation. Similarly, rats tested 3-7 weeks following exposure to 1.0Gy of ^{56}Fe particles failed to acquire an amphetamine-induced CPP (Rabin et al., 2000b). The results of these experiments indicated that exposure to heavy particles could disrupt the acquisition of a range of behaviors that are dependent upon the integrity of the dopaminergic system. Because of the possibility that there could be behavioral recovery of function in absence of neurochemical recovery, a group of rats was subjected to CTA training 16 weeks following irradiation. Compared to the non-irradiated controls, there was an apparent enhancement of the amphetamine-induced aversion in the rats exposed to 1.0 Gy of ^{56}Fe particles (Rabin et al., 2000a). Because these results were not predicted, it was felt that the experiment must be replicated to verify their validity. In addition, the observation of similar effects with a different dopamine-dependent behavior, the amphetamine-induced CPP would provide a measure of convergent validity for the result obtained with CTA paradigm.

The present paper constitute a review of some previous research on the effects of exposure to heavy particles on amphetamine-induced reinforcement and aversion, and a report of current experiments on the long-term effects of exposure on these endpoints.

METHODS

Subjects

The subjects for all experiments were male Sprague-Dawley rats weighing 200-225 g at the start of the experiment. Rats were exposed to 1.0 Gy of 1GeV/n ^{56}Fe particles using the alternating gradient synchrotron at Brookhaven National Laboratory. For exposure to heavy particles, the rats were placed in a well-ventilated plastic retaining tube, which restricted the movement of the rat. The tube was placed perpendicular to the beam and positioned to provide constant exposure of the rat's head, although other parts of the body were also exposed to varying lower doses of heavy particles. The characteristics of the beam have been detailed in a publication by Zeitlin et al. (1998). Controls were not exposed to the beam. Three days following exposure, the rats that were to by utilized in the long-term CTA studies and all rats for the CPP studies were returned to UMBC where they were maintained until behavioral testing.

Behavior

Two specific dopamine-mediated behaviors were studied using the dopamine agonist amphetamine: (1) CTA learning; and (2) the acquisition of CPP.

Taste Aversion Learning

A CTA is produced when a novel tasting conditioned stimulus is paired with an unconditioned stimulus. To produce a CTA rats were first adapted to a 23.5-hr water deprivation schedule on which they received water for 30 min/day for 5-7 days. On the conditioning day, the water bottle was replaced by a calibrated drinking tube containing a 10% sucrose solution and intake during the 30-min drinking period was measured. Immediately following the drinking period, the rats were administered the unconditioned stimuli, either injection of LiCl (2.2 mg/kg, ip) or amphetamine (3 mg/kg, ip). A control group was given an injection of isotonic saline, which would not be expected to affect sucrose intake in order to determine whether there were any spontaneous changes in behavior due to shipping or the passage of time. Twenty-four hours later, the rats were again presented with the calibrated drinking tubes containing 10% sucrose for 30 min and their intake recorded. The data for all experiments are presented as test day sucrose intake as the percentage of the conditioning day sucrose intake. A CTA is shown as a reduction in test day sucrose intake compared to conditioning day intake.

Conditioned Place Preference

An unbiased procedure was used to establish a CPP. On the first day of the experiment, the rats were given 15 min to explore an apparatus, which contains 3 distinctive compartments: large white and black compartments separated by a smaller gray compartment. On days 2, 4 and 6, half of the rats were given an injection of

amphetamine (2 mg/kg, ip) while maintained in a black compartment for 30 min; on alternate days (days 3, 5, and 7), the animals were given an injection of saline in the white compartments of the box. The pattern on injections was reversed for the remaining rats, which received the amphetamine injection in the white compartment and saline injection in the black compartment. On day 8, the rats were tested for the development of a CPP by being placed in the neutral gray compartment and allowed to move freely between all three compartments of the apparatus for 15 min (900 sec). The amount of time spent in each compartment was recorded. A CPP to amphetamine has developed if the rats spend significantly more time in the amphetamine-paired compartment than in the saline-paired compartment.

Statistical Analysis

Statistical analysis of the results was preformed using two-way analyses of variance. Post hoc comparisons between individual groups were performed using the Tukey-Kramer procedure to compensate for the multiple comparisons.

RESULTS

Taste Aversion Learning

As shown in Fig. 1, LiCl produced equivalent taste aversions in both the irradiated and control rats. The analysis of variance indicated that at none of the delay intervals was there a significant effect of treatment condition (radiated vs. control) on the acquisition of the LiCl-induced CTA ($F [1,47] = 0.13, p>0.10$). The main effect for delay interval for the comparison between the different conditioning days was significant ($F [2,47] = 7.19, p<0.01$). However, because the interaction was not significant ($F [2,47] = 0.46, p>0.10$), the decrease in sucrose intake was same for both the radiated and control rats.

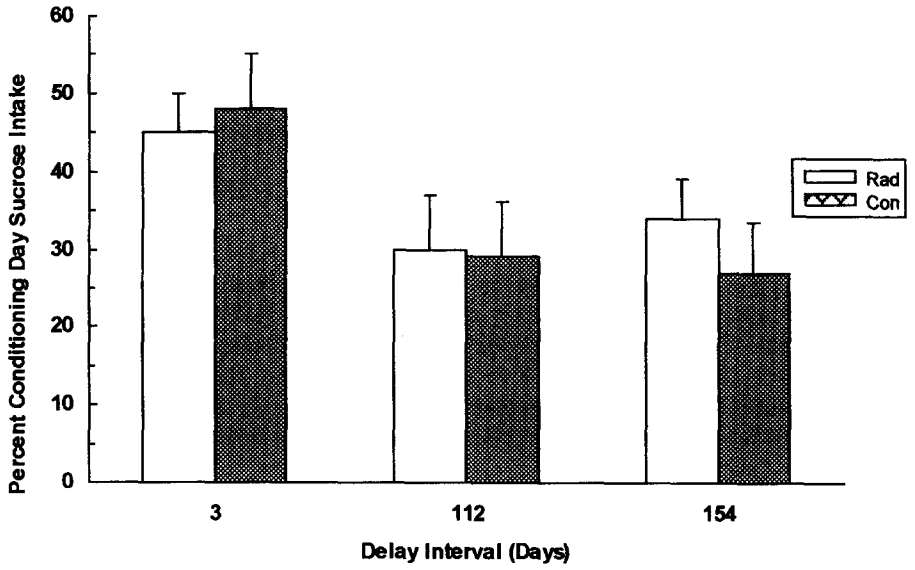

Fig. 1. Effects of exposure to 1 Gy of 1 GeV/n ^{56}Fe particles on lithium chloride-induced taste aversion learning as a function of the interval between the day of exposure and the day of conditioning. The data for the 2- and 112-day intervals has been redrawn from Rabin et al. (2000 a, with permission).

In contrast to the results with the LiCl-induced CTA, exposure to 1 Gy of 1 GeV/n ^{56}Fe particles had significant effects on the acquisition of an amphetamine-induced CTA (Figure 2) which depends upon the integrity of the central dopaminergic system. Compared to the control rats, the irradiated rats showed changes in amphetamine-induced CTA learning as a function of delay interval ($F [2,55] = 21.28, p<0.01$). The interaction between treatment condition and delay interval ($F [2,55] = 6.90, p<0.01$) was also significant, indicating that the

acquisition of an amphetamine-induced CTA varied as a function of the delay interval. Individual comparisons using the Tukey-Kramer procedure indicated that the sucrose intake of the rats conditioned 112 and 154 days after irradiation was significantly less than that of the rats conditioned 3 days following irradiation. Comparisons of the radiated animals with controls however, showed that only the rats conditioned 3 days following exposure showed a significant difference from their respective control group.

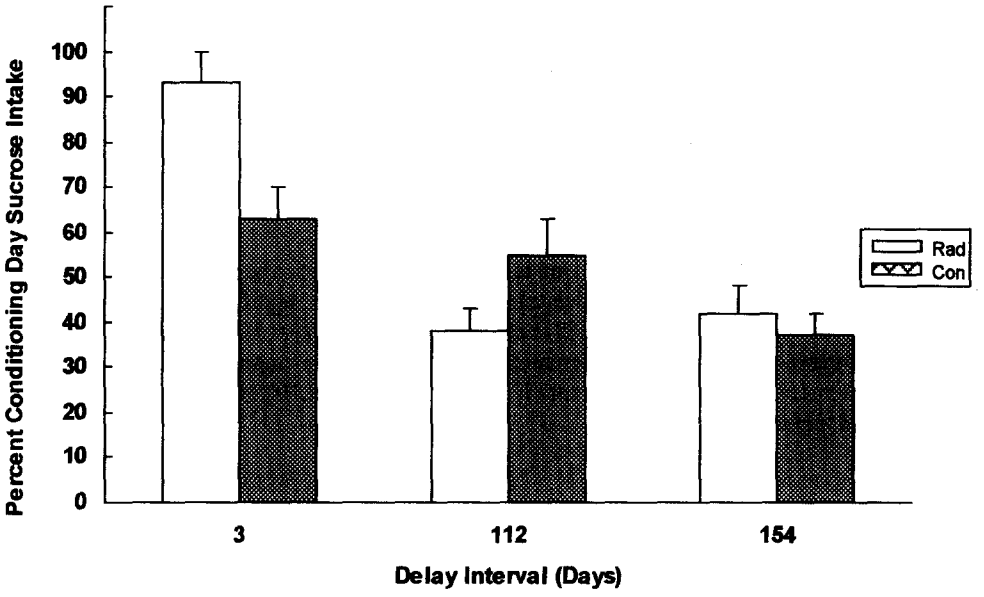

Fig. 2. Effects of exposure to 1 Gy of 1 GeV/n ^{56}Fe particles on amphetamine-induced taste aversion learning as a function of the interval between the day of exposure and the day of conditioning. The data for the 3- and 112-day intervals has been redrawn from Rabin et al. (2000 a) with permission.

As a control for the shipping to UMBC and for the delay between exposure and testing, some rats in each group tested 112 or 154 days following exposure to 1 Gy of ^{56}Fe particles (1 GeV/n) or to control procedures were administered equivalent amounts of physiological NaCl (0.9%) on the conditioning day (data not sown). Both the irradiated and control rats given an injection of NaCl on the conditioning day showed increased test day sucrose intake. This observation is consistent with previous reports that injection of this dose of NaCl does not produce a CTA (Rabin and Hunt, 1986; Riley and Tuck, 1985). As such results indicate that shipping and the delay interval did not affect CTA learning by themselves.

Place Preference Learning

The acquisition of a conditioned place preference in non-irradiated control rats is shown in Figure 3. At each time interval (3, 7, or 16 weeks), the control rats spent a significantly greater amount of time in the compartment that had been paired wit injection of amphetamine than in the compartment that had been paired with saline injection ($F [1,17] = 124.19$, $p>0.001$). Neither the main effect for the time of testing ($F [2,17] = 1.78$, $p>0.10$), nor the drug by time interaction ($F \{2,17] = 0.50$, $p>0.10$) was significant.

As shown in Figure 4, the rats exposed to 1 Gy of 1 GeV/n ^{56}Fe particles failed to acquire a conditioned place preference in response to injection of amphetamine at any time interval. Although the rats apparently spent slightly more time in the amphetamine-paired compartment than in the saline-paired compartment, neither the main effect for the comparison of the amount of time spent in the amphetamine- versus saline paired compartment nor the interaction with time of testing was significant ($F [1, 35] = 2.10$, $p>0.10$; $F[2,35] = 0.19$, $p>0.10$; respectively).

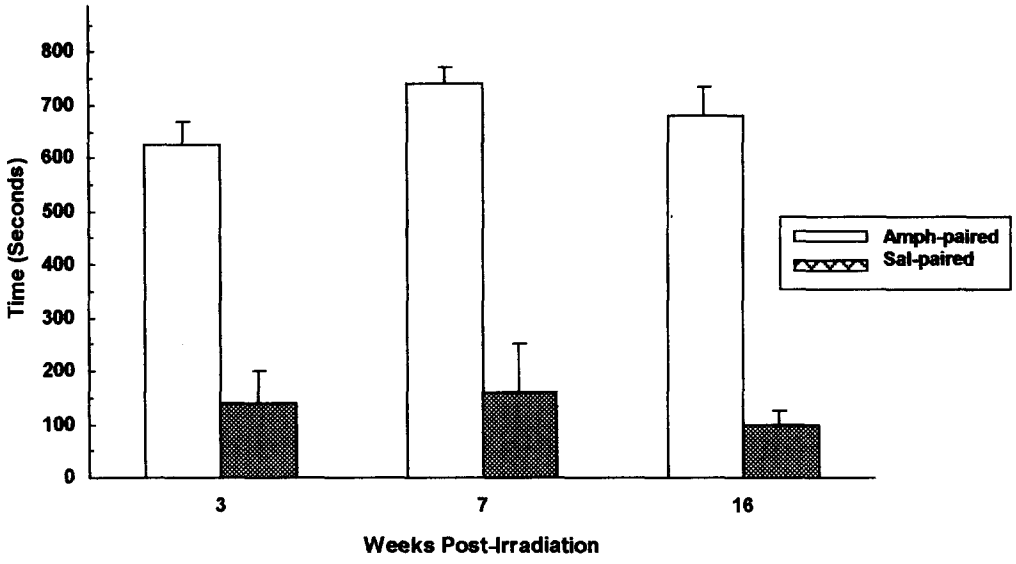

Fig. 3. Time spent in a compartment paired with amphetamine or saline injection in control rats as a function of time between control radiation procedures and conditioning. A conditioned place preference is shown by the significantly greater amount of time spent in the amphetamine-paired compartment.

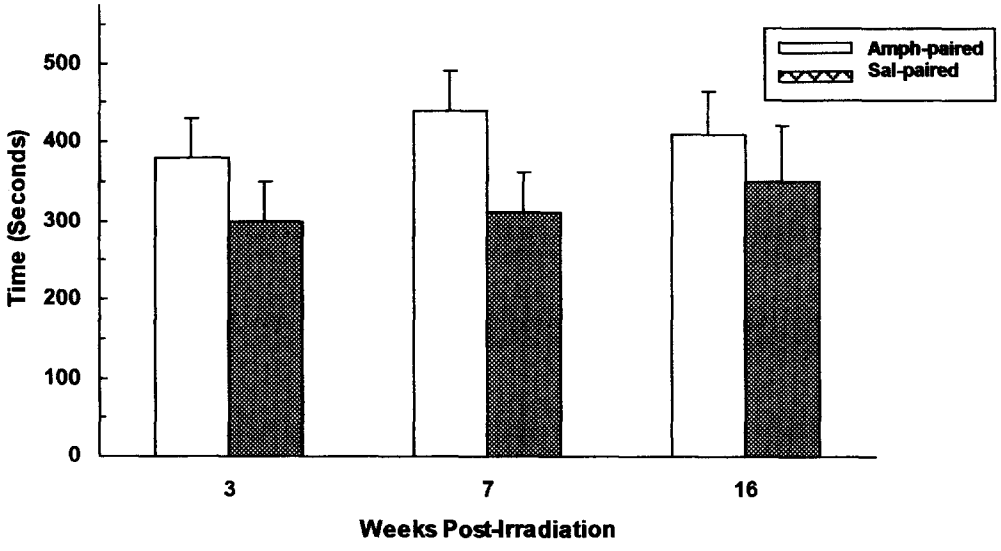

Fig. 4. Time spent in a compartment paired with amphetamine or saline injection in irradiated rats as a function of time between exposure to ^{56}Fe particles and conditioning. The lack of a conditioned place preference is shown by the observation of approximately equal amounts of time spent in the amphetamine- and saline-paired compartments.

DISCUSSION

Pharmacological studies have established that the acquisition of an amphetamine-induced CTA is dependent upon the integrity of the central dopaminergic system (Rabin and Hunt, 1989). Treating rats with D2 antagonist haloperidol prevents the acquisition of a dopamine-mediated CTA produced by injection of amphetamine, but has no effect on the CTA produced by injection of LiCl which is not mediated by the central dopaminergic system (Rabin and Hunt, 1986; Smith, 1980). Similarly, exposing rats to heavy particles (^{56}Fe, 600 MeV/n or 1 GeV/n) also disrupts the acquisition of an amphetamine-induced CTA without having any effects of LiCl-induced CTA learning (Rabin et al., 1998; 2000a). The observation that exposure to heavy particles can affect behaviors mediated by the dopaminergic system suggests that exposure to ^{56}Fe particles can affect a range of behaviors because the dopaminergic system plays a key role in the reinforcement of behavior (Nakajima, 1989; Benninger and Miller, 1998).

The data presented above indicate that exposure to 1 Gy of ^{56}Fe particles affects the acquisition of an amphetamine-induced CPP, which also is dependent upon the integrity of the dopaminergic system. Thus, exposure to these particles can affect the mechanisms of reinforcement. In addition, this represents a relatively permanent change in behavior because exposure to 1 Gy of ^{56}Fe particles produced equivalent disruptions of an amphetamine-induced place preference when tested 3, 7 or 16 weeks following irradiation. For this behavior, at least, there is no recovery of function within the period of time explored.

With regard to the long-term effects of exposure to ^{56}Fe particles on CTA learning, the data are less clear. The current experiment failed to replicate the results of a previous experiment (Rabin et al., 2000a) which suggested that there was an enhancement of the amphetamine-induced aversion 112 days following exposure to ^{56}Fe particles. While there was a significant reduction in sucrose intake at 154 days following exposure in the present experiment, which was similar to that observed previously at 112 days following irradiation, the sucrose intake of the irradiated animals was not significantly less than that of the controls. These results may reflect the observation that there was a significant decrease in the sucrose intake of the non-irradiated controls over time for both the amphetamine- and LiCl-injected animals. Thus, it is possible that there is an interaction between the age of the animal at time of conditioning and its responsiveness to both the sucrose solution and the unconditioned stimulus.

In summary, previous research has established that the dopaminergic deficits produced by exposure to ^{56}Fe particles are present at least up to six months following irradiation (Joseph et al., 1992). The exact nature of the tissue damage following exposure to heavy particles remains to be established. The changes in dopaminergic function may not reflect specific neuronal loss, but may instead involve alternations the functioning of dopaminergic neurons. In this regard, it has been shown that exposing rats to ^{56}Fe particles can affect the activation of second messenger systems (Joseph et al., 1994; Billalobos-Molina et al., 1994) as well as changes in membrane structure (Joseph et al., 1999) which may also affect neuronal function. The present research indicates that these changes in neuronal function are accompanied by a range of behavioral deficits, including deficits in dopamine-mediated taste aversion and place preference learning, and that there is no recovery of behavioral function.

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